~~Department~~ Copy

THE USE OF PHYTOHORMONES IN FACILITATING THE CLONAL PROPAGATION OF ALFALFA, WHEAT AND OATS

Hazara Singh Garcha

Department of Field Crops

University of Alberta

April, 1938

THE USE OF PHYTOHORMONES IN FACILITATING THE CLONAL PROPAGATION OF ALFALFA, WHEAT AND OATS

Hazara Singh Garcha

Department of Field Crops

A THESIS

submitted to the University of Alberta

in partial fulfilment of the requirements for

the degree of

MASTER OF SCIENCE

This thesis represents only 40% of the total work undertaken for the degree.

Edmonton, Alberta,

April, 1938.

ero
38
6

TABLE OF CONTENTS

Page

THE USE OF PHYTOHORMONES IN FACILITATING THE CLONAL PROPAGATION OF ALFALFA, WHEAT AND OATS

Hazara Singh Garcha

INTRODUCTION

Growth is a fundamental phenomenon and is therefore very important in the whole field of biological investigations. In recent years, investigations have been directed toward discovering the rules and mechanisms of growth processes in plants. Lately it has been demonstrated that there are some active chemical substances variously designated as "chemical messengers", plant hormones, growth-hormones, growth stimulators, correlation carriers, etc., which are concerned with every phase of plant growth and development. Thus progress in the development of our knowledge of plant growth is rapidly increasing and a voluminous literature has grown around these "growth substances".

Careful investigations have been made by Went (54), Thimann (40), Zimmerman (58), Bonner (40) and Cooper (9) on the action of growth stimulating chemicals on stems, shoots, leaves and roots of various plants. Many responses manifested by plants have been reported, such as local acceleration of growth, inelastic extensibility of

the cell wall, curvatures of stems and leaves, systematic versus local responses, swelling and proliferation of treated tissues; cell division and induction of adventitious roots; retardation of root elongation followed by increase in diameter and production of adventitious roots.

From these various responses, two very important, useful and interesting conclusions can be extracted:-

The first is that even minute quantities of the active, synthetic compounds, with a definite constitution, have rhizogenous power such as roots on roots and roots on stems. The extent of growth, of root formation, etc. is directly dependent on the amount or concentration of auxin present. The activity of the auxins seems to be much more quantitative than qualitative. Thimann (45) has brought out the fact, substantiated by Grace (14), that quantitative differences in auxin sensitivity exist not only between responses of stems and roots, but between almost all different types of tissues.

The second point, equally important, is that undoubtedly the whole complex of the growth hormones is necessary for eliciting response from plants, yet the physiological as well as the anatomical conditions within the plant and the specific reactivity of the tissues acted upon, are also responsible for their ready response in initiating roots for propagation. Thus it is obvious that the success of the treated cuttings, etc. depends largely upon creating a suitable environment; for varying

environmental conditions change the capacity of plants to respond when treated with any of the several synthetic auxins. Plants in different stages of maturity or similar plants at different seasons of the year vary in their capacity to respond to treatment. The influencing factors such as humidity, temperature, aeration and light must be suitably controlled for the expression of the responses involving the organization of cells for the production of adventitious organs.

Thus the discoveries, in rapid succession, of "growth hormone" and of identity with one of the plant hormones of root formation so called "rhizocaline" have stimulated the interests of the plant physiologists, the plant breeders, the home gardener and the commercial grower, in the use of these synthetic auxins. These then led to a number of practical applications, particularly in the rooting of cuttings which were very much harder to grow, took longer previously and, also, which were unresponsive to other treatments. This made it desirable to investigate the problem of asexual propagation rather thoroughly on a wide variety of plants.

During the past few years, 32 growth-promoting substances have been reported. Among them are 14 organic acids, 11 esters, 4 unsaturated carbon containing gases and salts of the various acids. All of these have been found effective in eliciting responses from various plant species. Among these, approximately 85 genera, involving

several hundred species and varieties have been found to make satisfactory response when given the proper chemical treatment. It seems to be established that the right concentration and environmental conditions can bring about, to a great extent, the desired response from a plant.

The main objective of this investigation is to present results obtained from using three synthetic auxins, namely B-indolylacetic, B-indolylbutyric and L-naphthaleneacetic acid. These three organic synthetic auxins were prepared by Dr. Manske at Ottawa, Canada. They were used in all of these experiments and were applied to the cuttings of only three crop plants; alfalfa, wheat and oats by the solution method with the purpose of determining their practical values in propagation by cuttings or clones. These three plants are almost new in the list of hormonal experiments. Their possibility for clonal propagation by the applications of these three synthetic auxins had not been reconnoitered nor statistically determined. Present investigations on these plants are therefore new and are replete with many practical utilities in the future of plant breeding work and in the science of genetics. However, one must remember that although the goal of the agriculturist is to get tangible results, the best to be had for a given cost, yet to the scientist, all truths have ultimate worth, and all search for truth has an inherent validity transcending any economic considerations.

The following seem to be some obvious material advantages which can be derived from the results of these investigations.

(1) In order to investigate and make use of the effect of environmental factors on the hereditary constitution of a very few available alfalfa or cereal plants, one must keep the hereditary constitution constant. It is, therefore, highly desirable to make a few cuttings from each available plant and from these to produce new adequately rooted plants. This may be facilitated by the use of the synthetic auxin treatment, thus making possible an extensive crop of similarly constituted plants. It is, therefore, abundantly clear that this investigation is very useful to plant breeders and research workers.

(2) Rooting cuttings or clones seems to be necessary and highly desirable for increasing any lot of alfalfa or cereal plants where only a limited number of seeds are available. For instance, in plant breeding work, it is very often impossible to get a sufficient number of F_2 seeds for the production of adequate populations because of sterility and incompatibility. Thus it is possible to multiply F_1 plants by growing rooted cuttings and to increase the number of F_2 seeds.

(3) These investigations also indicate a method of maintaining unaltered stocks of specially valuable plants, for example, rare selections, a few hybrid plants

possessing desired characteristics, imported material whether homozygous or heterozygous, etc. Individual plants selected as parents in a given cross may be maintained asexually after the cross has been completed and the seeds matured. Moreover, by this method, the identical parent stock may be used for any number of subsequent crosses. If for any reason, more F_2 seeds are desired, the F_1 plants may be further propagated asexually. How can a superior strain of alfalfa be raised for a certain region and enough seeds be obtained for that locality to grow an alfalfa crop? The hardiness and well branched type of root system characteristic of certain alfalfa varieties can be combined with the higher yielding capacity of some other group through variety, hybridization, and the few selected plants may be increased by this method in order to obtain more seeds.

(4) One can also produce desirable material for cytological studies within a short time.

On the whole, the rooting of cuttings are very desirable to increase the lots without any genetic alterations in any plant material. It is likely that many more possible uses will suggest themselves as workers come to realize the importance of alfalfa and cereal cuttings.

It is worth while to mention here that cytological and histological studies were not attempted in this work. The important researches of Kraus, Hamner

and Beal (15) of the University of Chicago are expected to reveal the true nature of these roots on cuttings in the very near future.

HISTORICAL BACKGROUND AND LITERATURE REVIEW.

While there are numerous references in the literature to the "Avena coleoptile", a "botanical guinea-pig", as it were, practically nothing has been published regarding the rooting of cereal and alfalfa clones.

References to the development of the rooting of cuttings are widely scattered through various journals and in many cases are merely short paragraphs in a publication dealing primarily with other problems such as the "avena test", the mechanism of the production and of the action of auxins and stimulation of cambial activity. This being the case, the author does not claim to have exhaustively searched such literature but feels sure that the more important papers dealing with the given problem have been consulted.

Let us begin from the beginning of the field of organogeny and connect what has already been done and studied and what are the problems at issue. Any investigator looks to the future as much as to the past. From time immemorial, thinking men always tried to explain why isolated pieces, twigs and leaves of many plants had regenerated and become independent individuals. This was an everyday phenomenon. What were the factors which set up regeneration and determined the kinds or organs and the manner of their formation? The physiologists of the day working with Bryophyllum crenatum whose detached

leaves develop roots and shoots, postulated the existence of "anlagen" (13). Later on, the investigators discarded the old view and hypothecated the correlation phenomenon. Duhamel (54) explained this by assuming two saps, one moving upward, the other downward from the leaves to the roots. Now if this downward stream were intercepted by any operation or any means, it caused callus formation and then from it root formation.

Sachs (32) laid great emphasis on the "special substances" theory. He postulated the existence of two different "stuffs," one heavier root-forming going downward, the other lighter stem-forming going upward. He called these two different actuating agencies as "Wuchsstoffe", the nutritive stimuli.

Goebel (13) rejected the view of energizing specific substances but placed emphasis on the nutritional factors and enunciated his theory of "attraction centres." He maintains that correlation, acceleration or inhibition phenomena of organs have a nutritional basis and are not a result of "internal secretion."

However, it was Loeb (26) who, working with the leaves of *Bryophyllum calycinum*, again stressed the theory of hormones. He suggested that a hormone plays a great part in the rooting of cuttings. The apical upper leaves on the horizontally laid stem sections, manufacture hormones which move downward through the cortex and accumu-

late at the lower side which then grows longer than the upper and which develops roots. He thus interconnected the phenomena of root formation and tropistic curvature of the stem-pieces.

This theory of tropisms played an important part in the development of the newest views on growth. It has stimulated the investigations of phytohormones which under certain conditions, not only affect growth, but cell division and determines some morphogenetic processes.

It was left for Went (54) to give the final convincing evidence for the existence of Sachs' postulated "Wuchsstoff". Went and van der Weij (54) then developed an exact technique of quantitative assay of the concentrated purified substance. They measured its activity in terms of the curvature of Avena coleoptile developed. When Went applied the extract from the Papaya leaves mixed with agar, to the base of the Acalypha cuttings, more numerous roots developed than otherwise. When he removed the buds from Pisum seedling stems and then applied auxin solution, a very small number of roots developed. He established then that the plant hormone which is produced by leaves and also by buds, has the power, somehow or other, to stimulate stem cuttings to initiate roots. Molisch (54) also substantiated that idea.

The promotion of root formation on cuttings as well as on intact plants by auxins, has been studied in

about 85 genera of plants (60) by investigators belonging to eleven or more nationalities, (54). Their researches which are going to be reviewed, demonstrated that growth substances not only hasten rootings of the cuttings of ordinary plants, but stimulate those plants which would otherwise never strike root and those which would have had only a meagre supply of roots.

Zimmerman and Hitchcock (58) prepared many chemical growth substances and compared their effectiveness in the initiation of roots on the cuttings of Vitis spp., Ilex, Hibiscus, Acer and many others. They used these compounds in aqueous solutions on cuttings as well as in lanoline paste on the intact plants. Their studies pointed out that B-indolylacetic, B-indolylbutyric and L-naphthaleneacetic acids are most effective over a wide range of concentrations, viz., ten to twenty times when the period of treatment varied from 6 to 90 hours. They suggested that the different species of plants vary in their sensitivity to the different amounts of auxin.

Cooper (9) working with the Eureka lemon tree, Acalypha, Lantana and Tradescantia, has obtained good results. His cuttings, being 5" long with leaves attached, developed better and more numerous roots when treated with .5 mg./1 gm. indolylacetic paste. His results indicated that the hormones not only increased rooting with cuttings that will not root when untreated but also caused the forma-

tion of roots on leafless lemon and Tradescantia cuttings which do not ordinarily form roots.

In the other paper (10) he pointed out that the solution method is more effective than Laibach's lanoline paste method in initiating roots on the cuttings.

Gocolasvili and Maximov (12) worked with Poncirus trifoliata and Citrus Unshiu, the latter does not root at all with ordinary methods. The lower parts of the cuttings were immersed in an aqueous solution of the B-indolylacetic acid (1:1500----1:5000) for from one to five days. The cuttings were then planted in a mixture of equal volumes of sand and peat moss. Much better results were obtained in the spring season when the conditions were more favorable than in winter. Their experiments with the trifoliate orange proved to be the most successful, about 100% having rooted within the short time of 30-45 days. Moreover, they state that 1:5000 concentration used on cuttings for 24 hours is equal to 1:2500 for 12 hours and resulted in 100% rooting. A longer period of treatment of both these solutions produced a toxic effect---cuttings were slower to root and manifested signs of rooting. The cuttings which had not shed their leaves, showed a more rapid rooting.

Trureckaja (46) used Citrus Limonium in addition to gooseberry, apple and olive trees, etc. He states that the best concentration of heteroauxin for plants appears

to be 10-25 mg. per 100 c.c. of water when the immersion period is 48-56 hours. The heteroauxin is very effective when the temperature is 28-30°C.

Cooper and Manton (11) in 1937, got remarkable results with Chrysanthemum cuttings. They tested almost 50 varieties of this plant. The terminal cuttings were made about three to three and one-half inches long and treated from 3-12 hours in B-indolylbutyric acid with a concentration of 1:10000 (100 mg. in one quart of hot water). After the treatment, the cuttings were planted in sand in the greenhouse. After 25 days, the cuttings were examined. Their data showed that the treated cuttings produced far more roots per cutting than the untreated controls.

Grace (14) gave us the most important results on root stimulation by the application of the three synthetic growth substances: L-naphthaleneacetic, B-indolylbutyric and B-indolylacetic acids and obtained physiologic curves of response to different concentrations. He treated the seeds of different varieties with hormone dust (a mixture of synthetic growth substance plus a carrier). The advantages of this seed treatment are first, that as the seed swells in germination, the adsorbed hormone is made available gradually, and second, that the danger of overdosage is greatly reduced. When one bushel of wheat seed was treated with only one-half ounce of dust mixture (2 p.p.m. of indolylacetic and naphthaleneacetic acids), the root length

increased 102%. Similar results were obtained with barley and bean roots.

He also applied the hormone solution, together with Hoagland's nutrient solutions, to growing plants such as Tomatoes, Nasturtiums, Salvia and Petunias. As little as 1/100 p.p.m. of L-naphtholeneacetic acid and 1/20 p.p.m. of B-indolylbutyric acid solutions stimulated the plants greatly.

Some 4000 cuttings, representing seven species, have been treated with hormone dust. The lower ends of the cuttings were dipped in the dust, the excess shaken off and the cuttings planted directly. His results indicated a very high degree of stimulation. Even the lower plant forms, expecially baker's yeast, were stimulated to a remarkable extent and gas production was greatly increased.

The main conclusion which we can draw from Grace's experiments is that if the proper concentration of the auxin solutions is used according to the sensitivity or tolerance of the tissues reacted, there will be no inhibitory effects, as some people claim, but always a stimulatory influence on the organs of the plant.

Grace's experiments suggest many useful applications of the hormone solution: Wilted lettuce or other plants and cut flowers can be made to regain their turgor and maintain their freshness over a longer period. The response of lower plant forms to the synthetic auxin

solutions, suggest their possible utility in industrial processes and fermentation.

It is appropriate at this place to explain a little how root production at the base of a cutting is stimulated by the synthetic auxins.

Snow (quoted in 54) and Went (54) postulated the theory of interlocking factors. A long time ago Blackman pointed out that growth was controlled by limiting factors. This has been again substantiated by the recent discovery of auxin, aneurin, biotin and sugar made by Went, Bonner and Warner (55). It is assumed that the main secondary function of auxin is to control or activate the movement of (54) or "suck" in (27) the other food factors. Rhizocaline (root-forming auxin), or one of Went's calines (52), seems to be synthesized mainly in the terminal buds, also to a lesser extent, in the apical very young leaves during the process of assimilation by some photosynthetic mechanism. On the other hand the shoot or apex-forming substances are formed at the base. Those root-forming materials normally move downward in the stem to cause root formation at the basal node. when we make a cutting, we may deplete the rhizocaline supply along with the discarded portion of the plant. But when the synthetic auxin is again supplied, as it were, though artificial in nature, is created.

It appears from the numerous localized responses of the experimental plants that these local growth reactions

involve a co-operative chain of a number of effective and essential chemical compounds. Thus auxin seems to act by affecting the transport of the other factors so that they become accumulated at the point of highest auxin concentration (54). If one is missing, there can be no growth. But now these synthetic active substances are used as substitutes for the missing links at the base. Then these synthetic auxins draw in rhizocaline auxin from the top downward to the base where it acts and induces roots. Pfeiffer (34) clarifies the situation through her microscopic studies of Cissus-sicyoides roots. Auxin, somehow or other stimulates the periclinal divisions in the pericycle which increase the number of layers lying over the xylem. Subsequent divisions, both periclinal and anticlinal produce small masses of tissue over the protoxylem points. These are the root primordia which come out laterally over the surface and appear as roots.

This explanation may not be true in all cases. However, the detailed histological studies of the dicotyledonous plants by Hamner (15) as well as monocotyledonous plants by Beal (15) will throw more light with reference to the root initiation by synthetic auxins.

This review reveals a very important struggle of how the minds of the investigators from the 17th century to the present continued to learn more and more about individual plants or parts of plants and integrated all the bits of

knowledge to build up a pyramid of conceptions, mostly around the "coleoptile stump", of the growth stimulators.

PRELIMINARY STUDIES.

INTRODUCTION.

J. Loeb (25, 26, 27,) was the first to suggest that leaves on the apical part of a cutting exercise a great influence on the development of roots. van der Lek (49) pointed out that nodes of a cutting differ in regard to the number of "root germs" and that there is a decrease of these rooting germs from the base to the top of a branch. That is the reason perhaps why a higher percentage of roots developed on the lower parts than on the higher. Thus differences arise in rooting capacity of the cuttings of the same branch. Went (50) demonstrated the existence of the growth-promoting substances. Zimmerman and Hitchcock (58) , Cooper (9) and Grace (14) have recently discovered that many auxins have the capacity of inducing root generation when they are applied to the cuttings.

A good deal of investigational research has been done during the last three years on the rooting of hardwood as well as softwood cuttings of various herbaceous plants and trees and shrubs. But little attention has been given to the propagation by cuttings of field crops, such as alfalfa, wheat and oats.

It has been demonstrated that synthetic auxins vary to a great extent in their power of eliciting responses from the various species of plants. One cannot

apply the same information obtained from experiments on one species of plants to others. Moreover, as different species of plants differ in their hereditary constitutions, it is obvious that they are likely to differ in their sensitivities to growth substances. A proper concentration for one plant may be an overdosage for another.

During the present investigation, there were not many sources of information as to optimum conditions of inducing rooting of leguminous and cereal cuttings. Burkholder (4) suggested to the writer that since not much work had been done on this line of investigation, one must try to feel his own way in the research. Besides, Koningsberger (19) pointed out that not very much was known along this line of work in his laboratory.

Accordingly, the following preliminary studies were directed toward the discovery of the best method to be applied and the most suitable environmental conditions to be provided the cuttings.

The main factors to be considered in the preliminary studies were:-

1. The stage of growth of the parent plant from which the cuttings should be taken.
2. The proper place for the basal cut; whether 5 m.m. below the node, just at the node or above the node.
3. Suitable synthetic auxins, their proper

concentrations and exact duration of treatment.

4. Proper rooting medium, whether sand or sand plus peat.
5. Optimum temperature and humidity.
6. One-node versus two-node cuttings.
7. Effect of light or absence of light on the cuttings when under auxin treatment and when in a propagating medium.

With these points in view, the various trials were made and some of their results are presented in this section.

Alfalfa

Material

Four Grimm alfalfa plants about two to three feet in height were brought in from the field on September 30, 1937. They were cut back and placed under good greenhouse growing conditions. As these plants reached the stage when desirable cuttings could be made, about 300 cuttings were taken for the preliminary set of experiments.

The synthetic auxins used were: first, L-naphthaleneacetic acid, second, B-indolylbutyric acid and third, B-indolylacetic acid. These auxin compounds will afterwards be referred to as acid 1, acid 2 and acid 3, respectively. The rooting media were: (1) sand (2) a mixture of equal quantities of sand and peat.

Methods

There were about twenty to thirty stalks on a single alfalfa plant. These were cut and placed in a wide pan full of water. From them, two-node sections called two-node cuttings, were prepared under water to avoid any chance of air-bubbles obstructing the passage of the transpiration stream. In all these cases, the cut was made about 5 mm. below the basal node. All cuts were angular in order to expose more surface to the contact of hormone solution. One or more leaves, the rhizocaline-producing centres, were retained on a second upper node of a cutting in one lot, while on another, the leaves were removed. The length of the cutting was approximately 5" depending upon the length of internode. All the cuttings were inspected carefully to see that all the basal nodes were uninjured and without leaves.

Only acids 2 and 3 were used in this case. As these indolyl acids were not freely soluble in water, it was therefore necessary that stock solutions should be prepared by dissolving the given amount of crystals (say 30 mg.) in a few drops (five or six) of 95% of ethylalcohol. First, only 100 c.c. of distilled water was added, heated and finally the volume was brought up to the desired point (say 500 c.c.) in a measuring flask. From this stock solution which was 60 p.p.m., other

lower concentrations were obtained by diluting with distilled water. Thus any desired concentration could be made. A series of dilutions was prepared, ranging from 300, 200, 150, 100, 50 p.p.m., in separate small beakers to ascertain which concentrations were favorable and which might have toxic effects. The column of the solution in the beakers was about 3/4 inch to 1 inch in depth. This was considered enough to make contact with the basal part of the cutting.

The cuttings were next transferred from the water into beakers containing the test solutions. These cuttings were allowed to remain there from five to thirty hours with a cloth placed over them to lessen the deteriorating effect of the solar rays on the hormone solutions in the beakers.

All the cuttings were planted in moist, sterilized sand. About two-thirds of each cutting was submerged in the rooting medium under the glass frame. Care was taken to spray the cuttings periodically with a fine syringe to avoid wilting which is the most detrimental condition for the rooting of cuttings. The temperature was maintained at approximately 65° by a thermostatic control. Humidity (about 70%) was kept under and around the frame.

<u>Results</u>.

These cuttings were left in place for at least three weeks before they were taken out for examination.

Their general condition was observed from time to time. The first important thing observed was that the very succulent cuttings were shrivelled and dried up in the propagating medium while the more woody and rigid ones survived. It was some time before the cuttings struck roots and got nutrients from the soil. During that helpless period, the cuttings, if very soft, died. The 5 mm. of stem left on below the basal node of every cutting decayed and then became the attractive centre for microorganisms, especially the cotton-like larvae of Sciara spp. commonly called fungous gnats.

After about three weeks, all the cuttings were picked out one by one from the rooting medium and examined as to the percentage of the cuttings successfully rooted.

The cuttings developed only fibrous roots, which emerged from the base of the node. Some internodes developed roots also. Swellings (papillae) were observed on some of them. The roots seemed vigorous but were very brittle and very short.

The results which are recorded in Appendices I to V suggest that a concentration of 300 and 200 p.p.m. of these two acids appears to have been toxic while the lower concentrations were rather stimulating.

The cuttings were best rooted, which were taken, not from the very young, tender stems, but from fairly

mature, strong and rigid stems which were better able to resist isolation from the parent plant. Sand appears to have been more favorable than the sand plus peat mixture, (Appendices I and II) the latter harbouring more pathogens than the former. Moreover, acid 2 appears to have been more effective for the stimulation of root primorida than acid 3. Finally, it seemed that the lower concentrations, especially of acid 2, with longer treatment were most effective. (Appendices III and IV).

To substantiate this and other points, some other experiments were conducted. The results are tabulated in Appendix V. They indicate that the 50 p.p.m. and 30 p.p.m. of the acids 2 and 1 respectively were very effective when the treatment time was about 24 hours. Although the untreated cuttings (controls) also developed roots, still it was observed that the treated ones showed more numerous and more vigorous roots than those of the untreated. The treated cuttings developed roots even on internodes. Moreover, it was observed that the checks, which happened to have been planted in the comparatively darker places under the frame, showed a much smaller number of roots than those in the light, while the treated cuttings placed in the darker positions, developed more roots than those in the light. This indicates that the auxin in the treated cuttings became inactivated by light whereas natural

auxin in the untreated ones become more stimulating for the development of roots.

One-node cuttings were then made and were treated with the most favorable and effective concentrations and were planted in the same sand medium. After three weeks, they were examined. Most of them were "dampened off", others died because of no root development.

It is hard to draw any definite conclusion from one experiment. However, it appears that the tendency to develop roots is there. The cuttings wilted faster than two-node cuttings, because of much less supply of food and auxin in their very small lengths.

Some of the rooted cuttings from the different concentrations were photographed as shown in Figure 1. This illustrates the fact that different concentrations have different stimulating effects.

Figure 1: Alfalfa stem cuttings after 24 days.

No. 1 and 2--terminal, 50 p.p.m. of acid 1, 3 and 4 basal 50 p.p.m. of acid 2; 5 terminal, 50 p.p.m. of acid 2; 6 terminal, 30 p.p.m. of acid 1; 7 basal control, 8 and 9 terminal controls.

Explanation: No. 1 and 2 very high concentrations seemed to be rather toxic, still No. 2 developed roots. No. 5 and 6 appeared to be more stimulated than others. No. 7 developed roots but it soon died. No. 8 and 9 dried out because roots did not develop early enough to absorb nutrients. It is concluded that conc. 30 p.p.m. of acid 1 and 50 p.p.m. of acid 2 are most effective.

Observing the abundance of disease spreading organisms, it was considered advisable to sterilize the frames with a formalin solution (1:100). This was prepared and one gallon of solution for each cubic foot of soil was sprayed on and in the frames. The frames were tightly closed for 10 days. They were then flushed out thoroughly with tap water and kept partly open to let the air out. After four more days the new cuttings were planted in their statistically randomized positions in the frames. Three days after planting, they began to wilt and died off, but no more fungous gnats were found in the frames. After 24 days, they were examined and not a single cutting had developed roots.

Even after thorough flushing and aeration, some chemical (perhaps paraformaldehyde) left from the formaldehyde might have inhibited the root development.

The conditions of the plants or cuttings themselves might be responsible for the failure. Nurserymen suggest that it is very difficult to induce plant growth during this period, December 15 to January 15, which is the resting period for all plants, no matter how optimum the conditions one may provide around plants.

Wheat

Material

The F_1 plants from an Agropyron X Triticum cross were taken from the field and the cuttings were made as

before in the case of alfalfa. Moreover, some plants of the wheat strain "Canus" were also used in these experiments.

The synthetic auxins used were of the same kind as had been used for alfalfa.

Methods

The stock solutions of each of the three acids were prepared by dissolving 25 mg. of crystals in 1c.c. of 95% ethylalcohol, adding first only 200 c.c. of distilled water, heating the mixture and finally bringing the volume up to 500 c.c. in a measuring flask. From this stock solution of 50 p.p.m., a series of dilutions was made.

The wheat cuttings were prepared in the same way as the alfalfa cuttings. They were treated in the different concentrations for 16 and 24 hours, and then planted in sand.

Results

Twenty-five days after planting, the two-node cuttings were examined. The results are tabulated and shown in Appendix VI, A and B. Due to the small number of cuttings used, the results are inconclusive. They suggest, however, that the lower concentrations (10 p.p.m. of acid 3 and 40 p.p.m. of acid 2) stimulated root development.

The one-node cuttings were made only from the basal portions of wheat plants. They were treated with

50, 25, 15 and 10 p.p.m. concentrations of L-naphthalene-acetic acid for 20 hours to four days. They were then planted in sand. The results were negative as shown in Appendix VI-B.

Discussion.

The one-node cuttings died very rapidly and showed no sign of response. The following is suggested as a possible explanation for the lack of response by this material.

In these cuttings, there was neither bud nor leaf left on the above-ground parts. An axillary bud on a cutting, as pointed out before, strongly promotes root formation. As there was no bud present on these cuttings to form a necessary morphogenetic substance to induce roots on the basal node in the soil, they failed to develop roots and died.

It is interesting to note in this connection that an apical bud which is a nucleus, as it were, is not only the most active rhizocaline-producing centre, but also the prolongator of the life of the internode (54). Accordingly, it is advisable to use the longer cuttings with bud or leaves intact. Moreover, Hammerling's experiments on Acetabularia support the conclusion that the regeneration of isolated sections is proportional to the size of the isolated piece and therefore, to the amount of root forming substance present in the above ground buds and young leaves (quoted in 54).

Oats

Material

Common Victory oat plants, about one foot in height and about three months old, were used for the one-node, and two-node cuttings, and their basal node was the third node from the base of the oat plant.

The synthetic auxins used in this case were acid 1 and acid 2, the rooting media used were fine sterilized sand and water.

Methods

The cuttings were made just at the node with a sharp knife. Only one cutting could be made from these plants. Accordingly, the basal cuttings were used in this case.

The required dilutions were made from the stock solutions already prepared. The cuttings were placed in the particular concentrations. About 3/4 inch of the cutting was submerged in the solution. Treatment time varied, ranging from 20 to 72 hours.

Some of the cuttings were planted on December 25, 1937 in the sterilized sand in pots and others were transferred to beakers containing water.

Common nutrient solution (Appendix VII) was prepared and applied to the water cultures.

Results

All cuttings were examined about two weeks after planting. The results are summarized in Table I.

Table I

The effect of
L-naphtholeneacetic and B-indolylbutyric acid
on the rooting of oat cuttings made just prior to heading
and grown in sand.

Acid	Conc.	Treatment (hours)	No. of cuttings planted	No. of cuttings rooted
L-Naph.	40 p.p.m.	20	10	0
"	40 p.p.m.	40	10	4
"	40 p.p.m.	72	10	0
"	20 p.p.m.	20	10	6
"	20 p.p.m.	40	10	6
"	20 p.p.m.	72	10	0
"	10 p.p.m.	20	10	0
"	10 p.p.m.	40	10	0
"	10 p.p.m.	72	10	0
B-Indol.	40 p.p.m.	20	10	8
"	40 p.p.m.	40	10	8
"	40 p.p.m.	72	10	0
"	20 p.p.m.	20	10	8
"	20 p.p.m.	40	10	6
"	20 p.p.m.	72	10	1
"	10 p.p.m.	20	10	0
"	10 p.p.m.	40	10	0
"	10 p.p.m.	72	10	3
Control	-----	20	10	0

In the case of the culture solution tests the same acids with similar treatments were used as with sand cultures; but in this case, all the cuttings developed roots except the controls. In the beakers, roots became visible after a week.

In the nutrient solutions, roots developed much faster than in sand. Those cuttings which had not developed

[illegible]

[illegible]

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

[illegible]

[illegible]

roots in sand were transferred to the solution where they soon developed roots. Two of the rooted cuttings were transplanted into six inch pots containing sterilized field soil, while the others were discarded. These two plants along with those of alfalfa and wheat are shown in Figure 2.

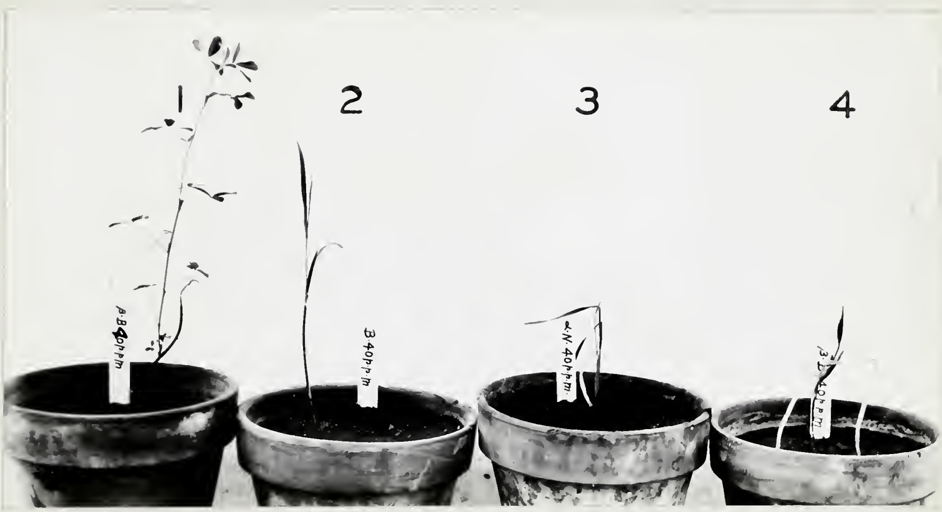


Figure 2. Plants after 45 days growth from wheat, oat and alfalfa cuttings.

No. 1: Alfalfa shoot 1.5 feet high from a rooted terminal cutting, treated with 40 p.p.m. and No. 2 treated with acid 2;and No. 3, treated with acid 1, on Victory oat plants. The former is much taller and more vigorous. No. 4, treated with acid 2 is a double-headed mature wheat plant, two leaves from the parent plant being still present. The indications are that synthetic auxins had a stimulating effect and that acid 2 is the more effective.

General Conclusions From the Preliminary Studies.

These preliminary experiments revealed several suggestive points. A great deal of useful information was obtained which was helpful in laying out further experiments. The main points disclosed are as follows:-

1. The basal surface of a cutting must be at the node. It is apparent that the basipetal polar flow of the rhizogenous substance may be interfered with at the nodal points, and it seems to accumulate there to some extent. If a cut is made just at the node, there is strong likelihood of the rhizocaline reacting at this point.

2. It is fairly clear from the results that B-indolylacetic acid is not effective in initiating roots while the other two are more stimulatory in this respect.

3. The wilting of the cuttings is a difficulty. It was found necessary to spray the cuttings at least three times a day.

4. Wheat seems to be a very unresponsive plant. But Graham and Stewart (54) state that if all the interacting factors are controlled, practically any plant can be induced to give 90% rooting from stem or leaf cuttings.

5. The two-node cuttings with buds or leaves intact give better results than those without them. One-node cuttings gave negative results.

FURTHER INVESTIGATIONS

Materials

Plants

i. Medicago sativa, var. Grimm.

ii. Triticum vulgare, var. Red Bobs.

iii. Avena sativa l. var. Victory.

Synthetic auxins

i. L-naphthaleneacetic acid. (acid 1).

ii. B-indolylbutyric acid. (acid 2).

iii. B-indolylacetic acid (acid 3, used in lanoline paste experiment only).

Alfalfa.

Experiment I.

Methods.

Grimm alfalfa was the only available material at the time when the present research was to be undertaken. It was therefore selected for the investigation. On January 3, 1938, five alfalfa plants were dug from a frozen field and brought to the greenhouse. They were cut back to about one and one-half inches from the crown and transplanted into boxes. Soil was firmly packed around the roots and from then on watered daily. As the greenhouse conditions were very favorable, numerous shoots soon arose and started growing very actively. In about three weeks, the stems had grown two to two and one-half feet in height.

Up to this time, preliminary studies had indicated the proper experimental conditions to provide the tolerance of the alfalfa plants to the different concentrations of the synthetic auxins and the magnitude of the response to be expected. Thus, the writer was fairly well equipped for conducting an experiment on alfalfa cuttings.

The majority of the stems were vigorous and in an active state of forced growth. On examination, it was discovered that only a small percentage had a large pith and were too soft. Most of them had reached a fairly mature stage and had small piths and were therefore hard. These stems, with buds and leaves, appeared to be in a suitable physiological condition as to cellular sensitivity and as to the presence of a suitable amount of the morphogenetic substance called rhizocaline. All this indicated that the stems were strong enough to withstand "cutting" shocks.

Of five plants, one was kept apart for a lanoline paste experiment while the remaining four were used for this cutting experiment. Numbers were given to them. Plant number three was not as vigorous nor as tall as the others. However, all were ready for cuttings.

Before describing further the technique of making the cuttings, it is now worth while to emphasize the fact that there are some implicit differences in every biological experimental material. These should be made explicit if possible. It is first necessary, therefore, to take

every variation due to both genetical and environmental differences into consideration and to include those detectable differences which one can estimate. But there are always some variations beyond human control which express themselves in the error of an experiment. It appears that there might be genetical differences among plants as well as physiological peculiarities among lower and upper parts of the same plant. van der Lek (49) often emphasized the fact that a number of "root initials" or the rooting capacity of the cuttings varies tremendously from the basal parts to the more apical parts of the same plant. It was necessary therefore to take two cuttings, one from the basal and one from the terminal part of each stem.

Twenty strong, fairly mature stems were selected from each plant and were cut above the ground and kept separate in four beakers containing tap water.

To make cuttings from alfalfa stems seems a rather simple matter, yet there are a few factors which must be considered. Previous studies have indicated that growing buds and young leaves on cuttings are essential for the initiation of the adventitious roots. Experiments of Zimmerman (59) have also shown that the roots can arise from any place on the cutting provided the cortical parenchymatous cells are present, whereas shoots arise only from the pre-existing buds which must therefore be retained

at the nodes of the cuttings. The preliminary tests have suggested that two-node cuttings are preferable to one-node cuttings in order to get satisfactory results.

Coming back to the alfalfa cut stems, there were four to five nodes left on each stem after the uppermost soft terminal tip was trimmed off. The basal cutting was made by cutting off the basal attached internodal part on the base of the stem. The stems were individually taken out from the large beakers and placed in an open water pan. The cuttings were made under water to avoid air entering the bottom cut ends. A short clean cut was made by inserting a sharp knife just at the node and then quickly slicing off the basal portion on a decided angle so as to offer the maximum surface for the rapid absorption of the solution. A clear sharp cut is necessary for rapid healing after the solution is absorbed. As soon as the cutting was made, it was quickly placed in the beakers containing water. Leaves from the bottom node were removed, while the axillary buds were carefully retained. But, one or two leaves on the upper second node of a cutting were retained. From one half to one inch of the internodal portion was retained above the upper node of a cutting. Similar operations were performed on the terminal cuttings. The length of the cutting was between four and six inches, depending largely on the length of the internode. By a similar method, forty

two-node cuttings (twenty basal plus twenty terminal) were made from each of the four plants. The cuttings from each plant were kept separate and treated according to the desired concentration of a given synthetic auxin for a certain number of hours. All cuttings were carefully inspected to see that all the bottom nodes bore uninjured buds and the basal cut surface was clean before they were placed. The preparation of solutions will be considered later.

The rooting medium was a sterilized medium fine, moist sand which provided a complex of conditions. Possibly a moist hard contact of the sand particles with the cuttings has an added stimulating thigmotropistic influence upon the initiation of the roots and upon the plant growth in general. The sand was about five inches in depth in the sterilized wooden flat which was about 20" by 24", large enough for 160 cuttings. Free circulation of air was provided for under the flats. The treated cuttings were taken out from the specified beakers and the definite labels with the randomized numbers, were attached to them. The numbers had previously been randomized on paper. The positions of the 160 cuttings were accordingly fixed in the flat which was divided into four equal portions six inches wide and twenty inches long. Then holes about one and one-half inches deep were made with a sharp drivel and cuttings were inserted into them. When the whole operation was

over, the holes were filled and the sand packed, taking care that the upright cuttings extended to the bottoms of the holes and could not easily be pulled out. Then the surface was levelled by watering. The fairly constant temperature of the greenhouse was about 69° F., while the relative humidity was about 65%. A diffused light was maintained for 24 hours so that the auxin might not be inactivated. After that, the usual greenhouse conditions prevailed. They were watered daily with a fine spray and sometimes with a fine syringe to avoid any injurious pressure on the cuttings. Care was exercised in not letting the cuttings wilt by frequent syringing to maintain the water content of the cuttings. They were left in the propagating medium for at least three weeks before removal for examination. Their general condition was observed from time to time. It seemed to be very satisfactory. The results will appear in the following section.

From the preliminary studies, it appeared that acid 1 and acid 2 were the two most effective auxins for the initiation of roots on cuttings. Their aqueous solutions were prepared as follows:-

Twenty-five mg. of acid 1 crystals were put in 100 c.c. of water and heated on a gentle flame to dissolve them. Raising to the boiling point would not affect the chemical. 400 c.c. of hot water were added to the solution to make up the required volume of 500 c.c. This was 50 p.p.m. stock solution. This was kept in dark brown glazed bottles which were placed in the refrigerator to

avoid deterioration. It was found that this solution could be kept in this way indefinitely.

The stock solution of acid 2 was prepared by dissolving 25 mg. first in a few drops of 95% ethylalcohol and adding only 100 c.c. distilled water, heating to drive off the alcohol[1] and finally bringing the volume up to 500 c.c. From this 50 p.p.m. solution, 30 p.p.m. dilution was also prepared. This solution deteriorates after about two weeks if it is not kept in a refrigerator. The deterioration is indicated by the brown coloration of the solution.

Ten different treatment solutions were prepared in ten separate small beakers which were marked with a red pencil. This experiment was designed to study the effects of the following variable conditions:-

1. Different plants.
2. Basal and apical cuttings of the same plant.
3. Two synthetic auxins: acid 1 and acid 2.
4. Two concentrations of each acid: 50 p.p.m. and 30 p.p.m.
5. Four replications.
6. Various interactions among the above factors.

One hundred and sixty cuttings were tied in bundles of four, each of which constituted a unit of treatment, and four bundles of each of the four plants were placed with their bottom ends in the ten solutions to a depth of 3/4 inch to 1 inch. The time of the treatment was 30 hours which was considered best from

1. See H.S. Tayler. A Treatise on Physical Chemistry. 523-524. 1930.

various angles.

After 30 hours, the cuttings were transferred to the rooting medium (sand) in the greenhouse.

Results.

There are various ways in which plants react to treatment. But in this case, only the actual number of roots developed on the cuttings was taken as the measure of response. The actual number of roots was therefore counted on each of the 160 cuttings. The data are shown in Appendix VIII, while Table II gives a picture of the significance of the factors involved. Table III shows the importance of the interaction of the various responses expressed. Figure 3 illustrates the fact that terminal cuttings are more stimulated than the basal ones, especially by the acid 2.

TABLE II

Analysis of Variance for Number of Roots.

Variation due to	D.F.	S.S.	Variance	F.		5%	1%
Parts	1	3330.63	3330.63	83.31	app.	3.94	6.90
Plants	3	465.43	155.14	3.88	"	2.70	3.98
Conc.	2	414.03	207.02	5.18	"	3.09	4.82
Replicates	#24	403.23	16.80	.42	"	----	----
Plants X Parts	3	542.2 2	180.74	9.52	"	2.70	3.98
Plants X Conc.	6	478.82	79.80	2.00	"	2.19	2.99
Parts X Conc.	2	17.62	8.81	.22	"	----	----
Parts X Conc. X Plants	6	1499.16	249.86	6 .2 5	"	2.10	3.98
Error	110	4397.87	39.98				
Total	159	11145.78					

S. E. $= \sqrt{39.98} = 6.32$

S. E. of treatment means $= 6.32/\sqrt{16} = 1.58$.

Minimum significance difference for treatment means =

$1.58 \times \sqrt{2} \times 2 = 4.46$.

Made up as follows: replicates in general, 3: replicates X plants, 9: replicates X parts, 3: and replicates X plants X parts, 9.

Table III

The effect of different concentration of two acids on the stimulation of root development of alfalfa cuttings.

cid	Concentration	Position of cutting	Mean (roots per cuttings)
	check	bottom	6.38
-naphthaleneacetic acid	30 p.p.m.	"	8.40
"	50 p.p.m.	"	4.80
-indolylbutyric acid	30 p.p.m.	"	10.40
"	50 p.p.m.	"	9.50
———	Check	top	15.00
-naphthaleneacetic acid	30 p.p.m.	"	18.40
"	50 p.p.m.	"	12.10
-indolylbutyric acid	30 p.p.m.	"	20.40
"	50 p.p.m.	"	19.30

Minimum significance difference 4.46

The stimulating effect of the auxins is obvious on the basal cuttings which have developed not less than 6.38 (check) plus 4.46 = 10.84 roots. Similarly it is apparent on the apical cuttings which have shown more than 15.8 (check) plus 4.46 = 19.46 roots.

Table IV.

Analysis of Variance for Acids.

riations due to	D.F.	Total sum of squares	Variance	F.	5%	1%
d	1	31.60	31.60	.8	--	--
ncentration	1	17.70	17.70	.5	--	--
ror	5	182.30	36.46			
Total	7					

Figure 3. Alfalfa rooted cuttings after 30 days in order of their degree of response.

Basal cuttings: No. 1 treated with 50 p.p.m. acid 1; No. 2 treated with 30 p.p.m. acid 2.

Terminal cuttings: No. 3, 50 p.p.m. of acid 1; No. 4, 50 p.p.m. of acid 2; No. 5, 30 p.p.m. of acid 1; No. 6, control; No. 7, 30 p.p.m. of acid 2; No. 8, 30 p.p.m. of acid 1; No. 9, 30 p.p.m. of acid 2; No. 10, 30 p.p.m. of acid 2.

The following general conclusions may be drawn:

1. The two acids show no significant difference when bottom cuttings are compared with controls.
2. L-naphthaleneacetic acid shows no significant difference even when the tops are compared with the check.
3. But, 30 p.p.m. of B-indolylbutyric acid does show a significant effect in stimulating the development of roots on top cuttings.
4. The checks themselves show a significant increase in roots when top cuttings are used instead of bottom ones.
5. Thirty p.p.m. of the L-naphthaleneacetic acid is significantly higher in the stimulation of roots than 50 p.p.m. of the same acid. It appears that this acid produces a toxic effect at the higher concentrations but is stimulatory at the lower limits such as 20 p.p.m. or 25 p.p.m. This appears to confirm, to a great extent , Grace's (14) "Physiological curves" for the three synthetic auxins. The optimum concentration of the B-indolylbutyric acid, seems to be about 50 p.p.m. This also agrees with Grace's results. Although these results do not show good agreement, still there is no significant difference between the effect of 30 p.p.m. and that of 50 p.p.m. of the acid. Thus it appears that the optimum point of second acid for the stimulation of roots is about 40 p.p.m.

Experiment II.

Methods.

The lanoline paste experiment, the material for which was provided by the fifth plant, as indicated above, was carried out in the following manner. Forty mg. of each of the three acids were placed in small pyrex beakers. A few drops of 95% of ethylalcohol were poured into each of them to dissolve the crystals. Ten c.c. of water were boiled very carefully to drive away the alcohol.

Twenty grams of lanoline (the same weight as that of water) were weighed and placed in three mortars. The 10 c.c. solution was poured in them. The beakers were rinsed with 10 c.c. more of hot water to make up 20 c.c. altogether. Now the mixture was triturated with pestles. The preparation turns from a yellowish tinge to a whitish coloration. Its concentration now is equal to 20 c.c. water plus 40 mg. acid. That is, the solution plus 20 gm. of lanoline results in the concentration of 1 mg. of auxin per gram of lanoline. Similarly another paste was prepared to obtain 2 mg. per 1 gm. of lanoline.

The third preparation was prepared rather differently. Three lots of three gm. of lanoline were put in three separate small mortars which were placed in a hot water pan to melt the lanoline. Then 90 mg. of each of the three auxins were put in three different mortars. Pestles were used to triturate them. These preparations were highly concentrated, being 30 mg. per 1 gm. of lanoline.

On February 27, 1938, the above three lanoline pastes were smeared with a tooth pick on different positions of the growing stems of the fifth alfalfa plant. A few punctures or longitudinal slits were made with a knife for the easy penetration of the paste. Smearing was repeated three times as the paste deteriorated on the plant which was exposed to light.

Results

After 12 days, marked swellings appeared in all cases except in controls. These proliferations formed at the points of application but 3% of L-naphthaleneacetic acid caused proliferation above the point of application. This acid 1 seems to be very effective in the lanoline paste method. The swelling on plant 3 gave rise to about 70 roots at one point while others were less marked. Acid 3 seemed to be very weak in proliferating roots on alfalfa plants. These points are well illustrated by Figure 4.

Figure 4: Alfalfa plants with roots after 24 days.

No. 1 control; No. 2 treated with .2% of L-naphthaleneacetic acid; No. 3, treated with 3% of L-naphthaleneacetic acid; No. 4 and 5, treated with 3% of B-indolylbutyric and B-indolylacetic acid respectively.

<u>Conclusion</u>:

About 3% of concentration of L-naphthaleneacetic acid seemed to be very stimulating for the development of roots on alfalfa nodes and also on internodes. Two-node or even one-node cuttings can be taken from these rooted intact plants and can be easily propagated.

Experiment III

Methods.

A third experiment with alfalfa was undertaken to determine the effect of aneurin on the responses of alfalfa cuttings to auxin. The alfalfa cuttings were made in the usual way. The cut was made just at the node in each case and the cuttings were made only from the apical parts of the alfalfa plants which were growing very actively. Besides a control, concentrations of 30 p.p.m. and 50 p.p.m. from each of two acid stock solutions were prepared. Cuttings were tied in bundles of five and transferred to the solutions with the basal ends submerged to a depth of three quarters of an inch. The treatment period given was 24 hours.

The treated cuttings were transferred to nutrient solutions immediately after the treatment. At the end of a week, about half of them from each bundle were transferred to the nutrient plus aneurin solution containing 1 mg. per litre. In this second transfer, only solution was used for the controls.

Results.

Figure 5, A definitely shows that B-indolylbutyric acid stimulates better root development than L-naphthalene-acetic acid. But when a very small quantity of aneurin was provided to the auxin treated cuttings, root length greatly increased as illustrated by Figure 5, B.

A B

Figure 5: Effect of aneurin on the auxin-treated alfalfa terminal cuttings.

A. Nos. 1 and 2 treated with 30 p.p.m. and 50 p.p.m. of L-naphthaleneacetic acid respectively; whereas Nos. 3 and 4 treated with 30 p.p.m. and 50 p.p.m. of B-indolylbutyric acid respectively; No. 5 a control;

B. Aneurin colution of 1 mg./1 litre conc. was applied to the above cuttings.

Conclusion:

Aneurin seemed to be very necessary for the elongation of roots which were initiated by the auxin solution.

From the results of these experiments, it appears that there is a difference between the stimulating effect of L-naphthaleneacetic acid and that of the B-indolylbutyric acid, but that difference is not statistically significant.

It is fairly clear from Appendix VIII, that, in some cases, a larger number of roots was formed by the untreated controls. It indicates that auxin was already there in excess. Moreover, some cuttings failed to strike roots even if the concentration was favorable.

It was surmised that when some additional factors are missing or of limiting quantity, plants would not develop roots, as Went et al. have pointed out in a recent article (55). It was thought better, therefore, to apply the lanoline paste directly to the nodes of the growing point so that roots might appear at those particular points and to make the cuttings when these latter had sufficiently developed.

Number 3 in Figure 4 shows a large group of vigorous stout roots far above the point of application. This fact throws some light on the movement of the applied auxin, Perhaps the transport is, to some extent, <u>acropetal</u>. It is hard to explain this result. However, it seems that auxin from the paste moved somehow to the transpiration path and flowed upward. However, this point is not clear.

The development of organs is not due to one specific factor, but it is the resultant of the whole complex interlocking system of limiting factors. "The activity

of any one factor" says Went (54), "is zero". Auxin, of course, causes root initiation but then other factors contribute their share of stimuli in the development of roots. Figure 5 shows the extent to which root length is increased by the presence of aneurin in the auxin solutions.

Finally, plants which were grown from the rooted cuttings transplanted in the field soil were darker green and more vigorous than those transplanted in sand and provided with sufficient nutrient solution.

WHEAT

EXPERIMENT I:

Method:

The Red Bobs variety of wheat used in this investigation is one of the standard varieties of wheat grown in Alberta.

Ten plump seeds were planted in each of three six inch pots, at five successive ten-day intervals, from October 2 to November 11, 1937. It was pointed out before that the reactivity of the tissues is a very essential factor to be taken into consideration in any auxin investigation. Keeping that point in mind, five batches of plants were grown on five different days in order to get experimental material having five different stages of growth, that is, five different physiological ages and their quantitative degrees of sensitivity. After emergence the seedlings were thinned to six per pot.

On January 20, when the cuttings were made, the first planting had reached the milk stage, the last two had not headed and the other three were at intermediate stages. Obviously, the plants differed in the lengths of the internodes. The first three plantings had four nodes above ground sufficiently widely spaced on each of the culms to make suitable cuttings. Whereas in the case of the plants which had not headed the internodes where so closely spaced that it was found necessary to include the tillering crown in the basal cuttings.

After first detaching the culms from the plants, the cuttings were made under water with a sharp knife so as to insure a clean and oblique severance at the node. Unfortunately, there was no leaf left on the upper node of the basal cuttings but one trimmed leaf was left on the apical ones. As soon as the cuttings were prepared they were placed in the water beaker; they ranged from four to eight inches in length.

As there were a few tillers developed on the crown nodes, these were cut off and kept separate from the cuttings. New tillers were used in another experiment in order to compare their root development with that of the cuttings.

Solutions of 30 p.p.m. and 50 p.p.m. of each of the acids were prepared from the stock solutions left over from the alfalfa experiments. Ten different concentrations were used in this experiment. Cuttings were tied in bundles of three, which was the unit of treatment, and were placed with their basal ends in the solutions to a depth of three-quarters of an inch to one inch. The time of treatment was 24 hours.

Steam sterilized sand, five inches deep in a flat, 25 inches by 30 inches, was thoroughly moistened and firmly packed. The appropriate labels marked with randomised numbers were attached to the 150 cuttings which were immediately planted in their proper places in three separate, longitudinal portions of the flat to a depth of about two inches. Provision was made for the free circulation of air beneath

the flat. After planting, the cuttings were sprayed with water. While light was obstructed to some extent, by a board in order to inhibit the inactivating effect of certain solar rays on the auxin solution (54), this precaution was observed only for 24 hours. The cuttings were sprayed three times daily. Temperature was kept at 65 - 70° F. on the surface of the sand, but at the bottom of the cutting in the sand, the temperature was about 60° F., which was rather low for the stimulation of the root development on the basal cuts (60).

<u>Results</u>

The previous studies indicated that the synthetic auxins have a stimulating effect on the rooting of wheat cuttings. Accordingly, the wheat cuttings experiment was laid out with a view to statistical treatment of the data. Both parts, bottom and top, were included in this experiment. Only three cuttings were used as a treatment unit. Twenty-nine days after planting in the rooting medium, the cuttings were examined. The following data were obtained:-

Table V

Effect of two synthetic auxins on the rooting of wheat cuttings.

	Conc.	Location of cuttings	Stage of growth	Number cuttings planted	Number cuttings rooted	Aver. No. roots
	Check	Bottom	III	3	1	2
B-indolyl-butyric	20 p.p.m.	"	I	3	1	1
	20 "	"	II	3	1	2
"	50 "	"	I	3	1	1
"	50 "	"	I	3	1	2
"	50 "	"	III	3	1	2
"	50 "	"	III	3	2	2
"	Check	Top		3	0	0
"	20 "	"		3	0	0
"	50 "	"	V	3	1	Primordia only
L-naphthaleneacetic	20 "	Bottom	II	3	1	1
	20 "	"	III	3	1	2
"	20 "	"	III	3	1	Primordia only
"	50 "	"	III	3	2	1
"	500 "	"	II	3	2	1
"	50 "	"	II	3	1	2
"	50 "	"	III	3	1	1
"	20 "	Top	II	3	1	Primordia
"	50 "	"	III	3	1	only

Note 1: I refers to the milky seed stage; II between blooming and milky stage; III to after-blooming stage; IV and V to the pre-heading stage.

Note 2: Treatment period in all above cases was 24 hours.

Conclusion

Although this table shows that no cutting developed more than two roots; still it suggests that the synthetic auxin solution has a stimulating effect on the root development especially on the basal cuttings. In the pre-heading stages of plant growth, the immature, soft plants showed no roots whatever. Fifty p.p.m. of the indolylbutyric acid seemed to be more effective in inducing roots than 50 p.p.m. of the other acid.

This experiment did not show as definite results as the alfalfa experiments did. The reason perhaps was that many of the cuttings dried out in the propagating medium as they were slow in developing roots. It may be that the stored-up food in the cuttings was insufficient. Possibly low humidity had something to do with it. Moreover, perhaps the auxin solution became inactivated at the cut surface of the wheat cuttings by some catalytic factors set free in the cutting operation and it might have played a part in causing the poor root growth (54). Lower temperature at the base of the cutting than at the upper, might have had something to do with the inactivity of the auxin. There might have been someoother supplementary factors which played their part in initiating or inhibiting root developments in the cuttings.

Experiment II.

Method

The lanoline pastes were prepared in the same way as in the case of the alfalfa experiments. The concentrations

of the pastes were 1 mg., 2 mg. and 30 mg. per 1 gm. of lanoline. These three pastes were smeared with tooth picks on different parts of wheat plants which were obtained from fellow investigators who were, at the time, working on other wheat projects. The temperature in the greenhouse was 69°F., while the humidity was rather low, about 40%. Old lanoline paste was removed from the culms and new dabs applied on three different occasions..

Result:

The results were negative in this case. There was only one case where a definite swelling occurred, whereas others showed only brownish spots at the point of application.

Conclusion:

It is very likely that these concentrations were not favorable for root development on the wheat plants, or it may be that those plants at that stage were not responsive to these pastes.

Experiment III:

Method:

Tillers were cut off from the wheat plants. The long leaves were trimmed off and the whole tiller was used as a cutting.

Three dilutions, 10, 15, 20 p.p.m. were made from the stock solutions of the same synthetic auxins already prepared. Treatment periods were 24 and 48 hours. Tillers were tied in bundles of four, which formed a unit of treatment. Thus seven bundles of fours were used in each acid, making 56 tillers in all.

All the tillers were taken out of the solutions at

the same time and labels with randomized numbers were attached. The tillers were then planted on January 21, 1938, in four inches of sterilized sand, in a flat 14 inches by 20 inches. Free air circulation was provided below the flat. The sand was levelled by thorough watering. Later the tillers were sprayed as in previous experiments. Figure 6I, A and B illustrate the stimulating effect of both of the acids. It also suggests that B-indolylbutyric acid is more effective than the other. Figure 7I also suggests that wheat tillers do show a response to auxin treatments.

Results:

The results are shown in Table VI.

Table VI.

Effect of the two synthetic auxins on the root development of wheat tillers. Four tillers were used in each treatment.

Acids	Conc.	Treatment (hours)	No. of tillers rooted	Aver. no. of roots
ɣ-indolylbutyric	check	24	2	2
	10 p.p.m.	24	1	3
	10 "	48	3	2
	15 "	24	1	4
	15 "	48	1	3
	20 "	24	1	3
	20 "	48	0	2
-naphthalene-acetic	check	48	2	1
	10 p.p.m.	24	1	1
	10 "	48	1	1
	15 "	24	1	2
	15 "	48	0	0
	20 "	24	1	1
	20 "	48	1	1

Conclusion:

Although Table VI does not show very striking results, it suggests that tillers are more easily stimulated to the development of roots than cuttings. It appears that possibly the tillers contain more food as well as more morphogenetic substance which induces roots than do the cuttings, and that they can withstand more adverse conditions.

Comparatively speaking, roots developed on B, Figure 6, shoots are sturdier and longer than on A, Figure 6, as shown below.

Figure 6: Stimulating effect of L-naphthaleneacetic acid (A) and B-indolylacetic acid (B) on the root development in wheat shoots grown in sand.

A. Stimulating effect of acid 1.

1.	20 p.p.m.	good roots but not so good as B - No. 1.
2.	15 p.p.m.	vigorous and numerous roots.
3.	10 p.p.m.	cutting died off.
4.	Control	not so healthy looking.
5.	Control	

B. Stimulating effect of acid 2.

1.	20 p.p.m.	good root system with a shoot.
2.	15 p.p.m.	no shoots.
3.	10 p.p.m.	good root system with a shoot.
4.	Control	good roots but not so good as 1.
5.	Control	died off.

Figure 7: Specimens representing effects of acids 1 and 2 on wheat tillers.

Figure 7:

No. 1: treated with 50 p.p.m. acid 1, two cuttings rooted.

No. 2: " " 30 " acid 1, two cuttings rooted.

No. 3: " " 50 " acid 2, two cuttings rooted.

A root developed from the third node, stimulation perhaps went upward. This was the best rooted cutting but it does not show in the photograph.

No. 4: treated with 30 p.p.m. and only two cuttings rooted.

No. 5: control, one cutting rooted. Only three roots developed and less secondary rootlets. The rest - 3 - died off.

Conclusion:

This comparison illustrates that the auxins do stimulate the root system of wheat plants. Although controls showed only 3 roots and fewer secondary rootlets, yet treated tillers showed more roots with more numerous rootlets, and developed more vigorous root systems.

Experiment IV.

Method:

According to the suggestion made by Went (54) as to the relative importance of aneurin application, a new experiment was conducted on March 16, 1938. Basal cuttings, from the second node above ground, were made from mature culms of wheat plants. Five cuttings in a bundle constituted the unit of treatment.

Dilutions of 15 p.p.m. and 30 p.p.m. were made from the stock solutions of the L-naphthaleneacetic and B-indolyl-butyric acids respectively. About 10 c.c. of these solutions

were used in glass tubes. The cuttings were placed with their basal ends in the solutions and treatment was continued for 30 hours.

The cuttings were then taken from the tubes and transferred to new tubes containing 10 c.c. of 1 mg. per litre aneurin solution. They were left here for five days and then nutrient solution was added to these as well as to the controls.

Results:

Twenty-five days after planting, the cuttings were examined. Observations suggested that when 30 p.p.m. of B-indolylbutyric acid was supplemented with aneurin, root initiation was greatly stimulated as shown in Table VII. It was also observed that aneurin induced lengthening of the roots to a great extent.

Table VII

Effect of auxin and aneurin on the rooting of wheat cuttings.

Auxins	Conc.	Number of cuttings planted	Number of cuttings rooted	Average number of roots
L-naphthalene-acetic acid	15 p.p.m.	5	0	Only brownish callus
B-indolylbutyric acid	30 p.p.m.	5	3	3
control	________	5	_	_

Conclusion:

This experiment indicates that a complex of factors does exist and plays part in the initiation of roots.

When some factors are missing from the chain of essential factors, roots may not develop. Another very important observation was made: B-indolylbutyric acid is far more effective in inducing root growth than the other acid, when it is supplemented with aneurin.

Oats

Experiment I.

Method.

Victory is a standard variety of oats, extensively grown in Alberta. It was therefore considered advisable to use this type for a test.

Strong, healthy looking seeds were planted in each of three six-inch pots, at five successive ten-day intervals from October 2nd to November 11th, 1937. These plantings were expected to yield sufficient material at each of five differential physiological growth stages. Nearly all the seeds germinated within eight days. These were thinned to six plants per pot.

As the last two stages were far behind the three earlier stages, attempts to make cuttings were delayed. However, when all were sufficiently mature, cuttings were taken from them on January 20th, 1938.

The cuttings were made in the same way as had been done in the previous cases of wheat and tied up in bundles of three to make a unit of treatment. Two dilutions, 20 p.p.m. and 50 p.p.m. were prepared from the stock solutions of each of the two acids mentioned in the previous experiments. Basal and apical cuttings were placed, at a depth of one inch in each of these four concentrations making eight treatments; one terminal and one basal cutting were also used as controls for each stage totalling ten treatments for 50 cuttings for each of the three replicates---------a total of 150 cuttings

in all. The treatment continued for 24 hours, temperature being 66° F.

After this treatment, randomized positions were fixed for each cutting in a flat, 25 inches by 30 inches, containing 5 inches of sterilized sand. The holes were drivelled and the treated cuttings were planted in them to a depth of about two inches, taking care that their basal ends touched the sand which had been firmly packed around them. The surface was levelled by watering and a canvas was placed over them to obstruct light for two days.

<u>Results</u>:

The planted cuttings were pulled up and examined after about a month. It was observed that the cuttings of the fairly mature oat plants seemed to respond to the auxin treatment more readily than those of wheat plants. The data derived are shown in Table VIII and stimulation is illustrated on Figure 8, Page 69.

Figure 8.
Effect of acid 1 and acid 2 on oat cuttings.

Nos. 1 to 5 treated with acid 1:-

1. 50 p.p.m.		died after root started.
2. 20 "		good roots and healthy looking cutting.
3. 20 "		more vigorous root growth.
4. 20 "		only tumerous growth or swelling developed.
5. Control	-	roots developed, but the cutting from above dried.

Nos. 6 to 11 treated with acid 2:-

6. 50 p.p.m.		killed off, only callus growth ensued.
7. 50 "		no root developed, but cutting was still fresh and green after 24 days.
8. 20 "		roots developed only from higher node (third node above ground) and plant was living.
9. 20 "		roots developed, then died.
10. Control	-	The cutting came off a hard, rigid plant.
11. Control	-	A few tumerous growths developed but no roots.

Table VIII

Effect of two synthetic auxins on the rooting of oat cuttings.

Acid	Conc.	Position cutting	Stage of growth	No. cuttings planted	Total No. rooted	Total No. roots
L - naphthalene-acetic	0 p.p.m.	bottom	II	3	2	2
	"	"	III	3	1	4
"	"	"	III	3	1	1
"	"	"	IV	3	1	2
"	20 p.p.m.	"	II	3	1	5
"	"	"	III	3	2	2
"	"	"	V	3	1	3
"	50 p.p.m.	"	L	3	1	2
"	0 p.p.m.	"	V	3	1	3
"	20 p.p.m.	"	V	3	1	2
"	50 p.p.m.	"		3	0	0
B -indolylbut-yric	20 p.p.m.	"	II	3	1	6
"	"	"	V	3	1	2
"	50 p.p.m.	"	II	3		primordia only
"	20 p.p.m.	top	V	3	1	2
"	50 p.p.m.	"	II	3	1	primordia only

Conclusion:

These results indicate that the effect of the auxins is to increase the number of roots when 20 p.p.m. of either of the two acids are used. The controls also produce a high percentage of roots.

On the whole this experiment suggests one possibility, viz. that the second node above ground is capable of giving

[illegible]

[illegible]

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

[illegible]

[illegible]

rise to roots which may be increased by the application of auxins. It is not advisable to draw any definite conclusion from one experiment with the limited number of cuttings used. However, there appears to have been a tendency toward a higher percentage of cuttings producing roots when the lower concentrations of L-naphthaleneacetic acid were used. Twenty-four hour treatment seemed to be more favorable than other longer treatments.

Experiment II.

Method

The same method was used as Expt. II with wheat plants.

Results

The results were negative.

Conclusion

Auxin failed to elicit any response from these plants probably because they were too advanced before beginning treatment.

Experiment III

Method

Tillers were collected from the oat plants and were used for Experiment III. There were 56 tillers, with long leaves trimmed off to decrease transpiration and thus to lessen the possibility of wilting. The same treatment was administered to these tillers as was given to the wheat in Experiment III. When the tillers were taken out of the solutions, the labels with randomized numbers were attached to them. They were placed in their respective positions and firmly planted in

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

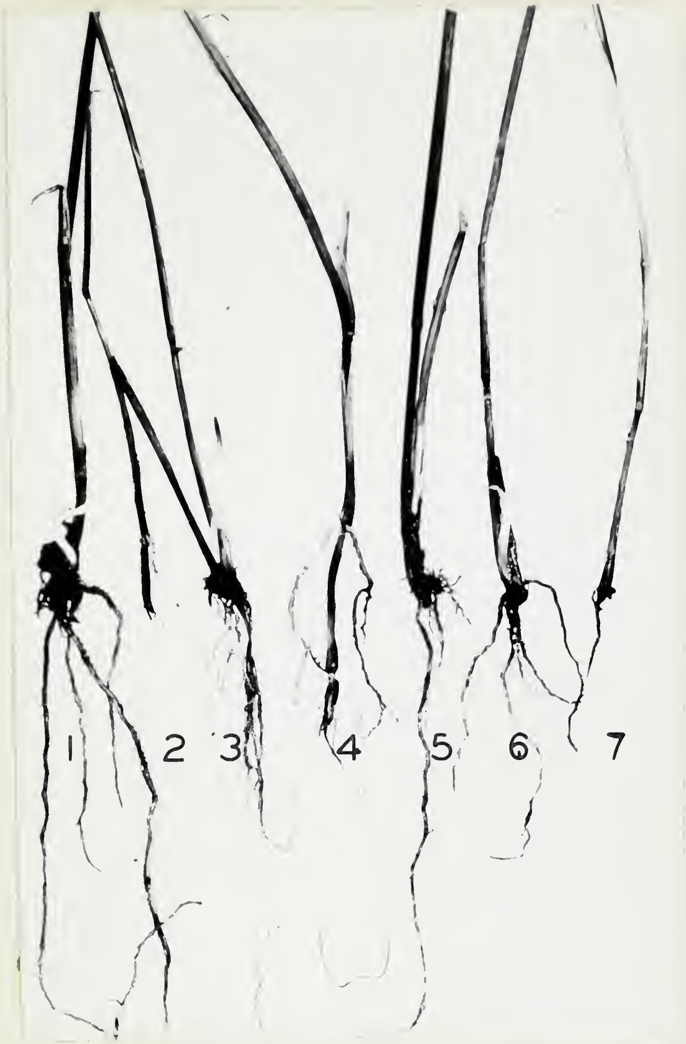
the sand. Diffused light was allowed for two days.

Results:

Tillers were examined after about a month. They showed a definite response to the treatments as shown by Figure 9. "A" shoots showed a more vigorous root-system than those of "B". It appears that B-indolylbutyric acid is rather more conducive to increase of root lengths than is the other acid. The results obtained are shown in Table IX.

A

Figure 9: Effect of L-naphthaleneacetic and B-indolylbutyric acid on the stimulation of the roots of oat tillers.



B.

Fig. 9: Effect of L-naphthalencacetic and B-indolylbutyric acid on the stimulation of the roots of oat tillers.

A. Nos. 1 - 7 treated with L-naphthalencacetic acid.

1. 2 0 p.p.m. good root system, shoots developed from the third node

2 . 2 0 p.p.m. rigorous root system with a good shoot.

3. 15 p.p.m. good root system with shoot

4. 15 p.p.m. very vigorous root system with two shoots.

5. 10 p.p.m. root system was fairly good but poor upper growth.

6. 10 p.p.m. good roots and one shoot on.

7. control - good roots.

B. Nos. 1 - 7. treated with B-indolylbutyric acid.

1. 2 0 p.p.m. vigorous root system with a shoot.

2. 20 p.p.m. roots developed from the third node.

3. 15 p.p.m . good root development with two shoots.

4. 15 p.p.m. roots developed from above the second node.

5. 10 p.p.m. good root system developed with three shoots.

6. 10 p.p.m. roots developed but only one shoot.

7. Control - roots developed but remained stunted.

Table IX

Effect of two synthetic auxins on the rooting of oat shoots.

Acid	Conc.	Treatment duration	Number shoots planted	Number shoots rooted	Number roots
	Check	24	4	1	4
L-naphthalene-acetic	10 p.p.m.	24	4	4	5
"	10 "	48	4	2	7
"	15 "	24	4	3	5
"	15 "	48	4	3	6
"	20 "	24	4	3	8
"	20 "	48	4	2	4
	Check	24	4	3	5
B-indolylbuty-ric	10 p.p.m.	24	4	1	6
"	10 "	48	4	2	3
"	15 "	24	4	2	2.5
"	15 "	48	4	3	7
"	20 "	24	4	1	2
"	20 "	48	4	1	4

Conclusion:

From the results, it appears that oat tillers respond better than cuttings to the auxin treatment. L-naphthalene-acetic acid is more stimulating than B-indolylbutyric acid in root development. Moreover, L-naphthaleneacetic acid appears to have a quicker action than the latter. The oat

tillers respond far better than those of wheat. Possibly the cells of oat plants are more sensitive to auxin treatments than those of wheat plants.

Discussion

If the evidence presented in the foregoing experiments is collected, there appears to be a few important points which should be stressed and considered in their relations to other known facts.

Table III definitely indicates that alfalfa is very sensitive to auxins, especially the terminal cuttings which seem either to have more root-primordia, already formed, or to be more strongly stimulated to produce new primordia than the basal cuttings. But van der Lek (49) suggests that there are more "root germs" in the lower nodes than in the upper ones. However, this seems not to be true in the case of alfalfa stems. Even the apical controls produce more roots than the basal ones. It is perhaps due to the fact that the apical cuttings, being younger and nearer to the auxin forming centres, get more rhizogenous substance than the basal ones. Moreover, it may be possible that in some way, auxin becomes more readily inactivated in the older basal cuttings than in the younger upper cuttings.

Two-node cuttings with leaves intact developed good roots, whereas one-node cuttings having no leaves showed no signs of roots whatever. This indicates that leaves do make root-forming substances which flow downward. Numbers 2 and 3 of Figure 4 and number 2 of Figure 5 suggest that auxin moved upward. It is possible that acid 1 at 3%

and 50 p.p.m. is capable of obstructing the normal basipetal movement of the rhizocaline which seems to be controlled above the point of auxin application. There it perhaps accumulates and stimulates root formation. Moreover, a bud at the upper node of a cutting, is very necessary for the production of roots. Perhaps this was the reason that bud-less one-node cuttings and internodes of these plants failed to root. This was also well substantiated by Molisch (quoted in 54).

All these results indicate the possibility of asexually reproducing selected alfalfa plants, and, to some extent, wheat and oats. The method suggested also makes possible the multiplication of populations without genetic alteration.

As to cereals, Table I and VII definitely show that oats and wheat cuttings do respond to the auxin treatments. These results suggest two very significant points. In dicotyledonous plants, the histogens of adventitious roots generally arise from the pericycle. But in wheat and oats neither a cambium is present to initiate callus formation (21) nor are clearly observable pericycle cells which the auxins usually stimulate to form, first root initials, then root primordia, and finally, roots (54). Probably the parenchymatous cells which are lying just outside the region of vascular bundles, may be stimulated to form roots (15). It may be that these auxins actually induce some cells of the

bundle sheath to produce root primordia (5). However, this whole mechanism is not clear as yet. The second suggestion is that as the axillary buds and embryonic leaves are not present on these cuttings, it is very difficult for them to produce enough aneurin and rhizocaline, which seem to be necessary complements for root growth. When a minute quantity (1 mg. per litre) of aneurin had been applied to the auxin treated wheat cuttings, roots did develop as shown in Table VII. In the future it will not be difficult to produce roots when rhizocaline (one of calines) will have been isolated and supplied to the cuttings at the same time as the auxin treatment is given (52, 53).

These experiments on cereals were preliminary in nature. On the whole those on wheat and oats were not satisfactory to the same extent as those on alfalfa, still a possible way has been pointed out by which to overcome the difficulty in these crop plants.

One observation of great practical importance and probable significance was made during the examination of the rooted cuttings of alfalfa, viz., that the treated cuttings developed larger nodules than the untreated ones. That was perhaps due to the fact that the added auxin accelerated the developing nodules which had been already initiated, perhaps by indolylacetic acid secreted by *Rhizobium leguminosarum* (44). There seems to be some

[illegible] to produce root primordia (?). However, this whole mechanism is not clear as yet. The second suggestion is that as the [illegible] buds and [illegible] leaves are not present in these cuttings, it is very difficult for them to produce enough auxin and carbohydrates, which seem to be necessary requirements for root growth. [illegible] (auxin) is [illegible] and [illegible] applied to the [illegible] cuttings, [illegible] as shown in Table VII. In the future it will not be [illegible] to produce roots [illegible] of [illegible] will have been [illegible] and applied to the cuttings at the same time as the auxin treatment is given ([illegible], 45).

These experiments on [illegible] were preliminary in nature. On the whole [illegible] satisfactory [illegible] alfalfa, [illegible] a possible way [illegible] the difficulty in [illegible].

The [illegible] and [illegible] significance [illegible] of the rooted cuttings of alfalfa, viz., that the rooted cuttings developed [illegible]. That has [illegible] the [illegible] isolated, [illegible] *Rhizoctonia* [illegible] ([illegible]). [illegible]

possibility of increasing nitrogen-fixation by a suitable application of this auxin treatment and thus to obtain larger yields of alfalfa crop.

It seems proper to draw attention to the differences between natural roots and roots induced by the synthetic auxins. Auxin-stimulated cuttings have only a fibrous root system; roots are often developed on internodes; they are more numerous and more vigorous, though shorter. They have a more rapid development but tend to be very brittle in comparison to the flexible root system of naturally grown plants.

In all these experiments, one fact stands out very strikingly, viz., that about 30 p.p.m. of L-naphthaleneacetic acid and 50 p.p.m. of B-indolylbutyric acid seems to be the optimum concentrations for root developments on the clones of these plants. This agrees with Grace's physiologic curves very closely to his results.

This investigation undoubtedly solves some problems but raises many more. First of all, it is not unproved now that auxin plays its main function in cell elongation. But it is still vague whether these synthetic auxins themselves are instrumental in bringing about root induction in cuttings or whether they simply indirectly activate or energize some other factors and substances which then stimulate roots. Another extremely difficult point which needs clarification is how and why the same synthetic auxins induce the production of adventitious

roots, yet inhibit their size. Why these auxin-produced roots are stout and sturdy while natural normal controls are thin and frail. It is hard to say whether the gene complex equipment is under the influence of these growth-hormones or vice versa. Is there any mechanism by which auxin can connect the sensitivity of the plant with the environmental conditions? These and many more fundamental questions strongly suggest how little we know of actual processes of plant growth and this mere fact throws a challenge to investigators to search for more facts. Accordingly, it necessitates opening up wider fields for research.

General Summary and Conclusions

The experiments showed the following facts:-

1. The following environmental conditions were found to be satisfactory:-
   - (a) Atmosphere humidity - 70%.
   - (b) Temperature - in the soil - 75° F.
     in the air - 70° F.
   - (c) Diffuse light for first 24 hours.
   - (d) Sand rather than mixture of peat and sand.
   - (e) Frequent spraying - at least three times a day.

2. The following points are to be considered when making cuttings:
   - (a) A cut should be clear, sharp and angular at the basal node of a cutting.
   - (b) Cutting should be made at the node to include the whole basal node where the root-forming substances seem to accumulate to some extent.
   - (c) Cuttings should have two nodes and should be four to eight inches in length. One-node cuttings generally die off fairly early because of their fast wilting before the roots proliferate and are able to absorb nutrients to maintain themselves.
   - (d) Leaves should be retained on the second upper node of a cutting.
   - (e) Apical rather than basal cutting of alfalfa is preferable, whereas the reverse for wheat

and oats is true.

(f) Parent plant should be mature and woody rather than pithy and soft.

Cereal tillers, small or large, are preferable to cuttings, since they have leaves and are comparatively longer.

3. The synthetic auxins used produced response in the following order of their effectiveness:-

(a) B-Indolylbutyric acid:-

dilution	45 - 50 p.p.m.	for alfalfa.
"	50 p.p.m.	for wheat and oat cuttings.
"	15 p.p.m.	for cereal tillers.

(b) L-Naphthaleneacetic acid:-

dilution	20 - 30 p.p.m.	for alfalfa.
"	10 p.p.m.	for wheat and oat cuttings.
"	10 - 15 p.p.m.	for wheat and oat tillers.

(c) B-Indolylacetic acid:-

Not very effective for inducing roots, hence not used much.

Note: It should be remembered that within limits the lower the concentrations used and the longer duration of treatment, the more effective the response will result. Generally speaking, the treatment time for the above concentrations should be 24 hours.

4. The following points are to be considered when (i) the lanoline-paste and (ii) auxin-aneurin methods are used:-

   (i) (a) L-Naphthaleneacetic acid 30 mg. per 1 gram of melted lanoline for developing roots directly on alfalfa plant.

   (b) B-Indolylbutyric acid - 30 mg. per 1 gram of melted lanoline for root initiation on alfalfa plants.

   (c) B-Indolylacetic acid being least effective.

   (ii) One mg. of aneurin per one litre of water to be used directly after the application of 30 p.p.m. of B-indolylbutyric acid on wheat cuttings.

<u>Note:</u> When these plants fail to produce roots by the aqueous solution method, attempts should be made to stimulate cuttings to initiate roots by means of the two following methods:

   (i) Lanoline-paste method - to obtain roots on the plant stems before cuttings are made.

   (ii) Auxin-aneurin method - to supply the cuttings with some substitute for a missing factor.

5. All the experiments suggest that growth is a combined expression of all the various integrated factors, processes and functions. A complex interlocking system of limiting factors appears to be at a basis of root production. Aneurin seems to be more limiting in root elongation than others.

ACKNOWLEDGMENTS.

The writer is especially appreciative of the generous assistance given him by Dr. K.W. Neatby, head of the Department of Field Crops, under whose guidance and encouragement, this investigation and the preparation of this manuscript have been carried out.

The author also wishes to acknowledge his indebtedness to Dr. J.R. Fryer, Dr. A.B. Brown and Dr. A.G. McCalla for many helpful criticisms and valuable suggestions offered during the course of study. As all the preliminary experiments were performed in the horticultural greenhouse, the writer wishes to make acknowledgement to Dr. J.S. Shoemaker and Mr. J.C. Greenough for their willing cooperation and for placing at his disposal many appliances necessary for the experiment.

The author wishes to extend his hearty thanks to Dr. P.W. Zimmerman for his suggestions given before and during the progress of the work.

REFERENCES

1. AVERY, G.S., Burkholder, P.R. and Creighton, H.B. Growth hormone in terminal shoots of Nicotiana in relation to light. Am Jour. Bot. 24(10 : 667-73 : Dec. 1937.

2. BONNER, J. Studies on the growth hormone of plants. V. The relation of cell elongation to cell wall formation. Proc. Nat. Acad. Sci. 20 (6) : 393-97 : 1934.

3. BOYSEN-JENSEN, P. Growth hormones in plants. McGraw-Hill Book Co., Inc., 1936.

4. BURKHOLDER, P.P. Private communication.

5. CARLSON, M.C. Origin and development of adventitious roots in cuttings. Contrib. Boyce Thompson Inst. 5 (3) : 313-30 : 1933.

6. CHOLODNY, N. Charles Darwin and modern Theory of tropisms. Sci. 86(2238) : 468-86: Nov. 19, 1937.

7. CHOLODNY, N.G. Growth hormones and development of plants. Nature 138: 586: 1936.

8. CONNARD, M.H. and ZIMMERMAN, P.W. The origin of adventitious roots in cuttings of Partulaca oleracea L. Contrib. Boyce Thompson Inst. 3 (3) : 337-46: 1931

9. COOPER, W.C. Hormones in relation to root formation on stem cuttings. Plant Physiology. 10(4): 789-94: 1935.

10. ------- Transport of root forming hormone in woody cuttings. Plant Phys. 11 (4) : 779-93: Oct. 1936.

11. ------- And Manton, J.B. Effect of hormones on the rooting of Chrysanthemum cuttings. The Florists' Review, July 19, 1937.

12. GOCOLASVILI, M.M. and MAXIMOV, N.A. Effect of heter-auxin in the rootage of cuttings from subtropical wood. Comptes Rendus (Doklady) de l'academie-des sciences de L' U.S.S.R. Vol. 17 (1-2): 51-54: 1937.

13. GOEBEL, KARL. Regeneration in plants. Bull. The Torrey Bot. Club. 30(4) :197-205:1903.

14. GRACE, N.H. Physiologic curve of responses to phytohormones by seeds, growing plants, cuttings and lower plant forms. Can. Jour. Res. C. 15:538-46; 1937.

15. HAMNER, K.C., University of Chicago, Private communication.

16. HITCHCOCK, A.E. and ZIMMERMAN, P.W. Effect of growth substances on the rooting response of cuttings. Contrib. Boyce Thompson Inst. 8(1):63-79:1936.

17. HUXLEY, J.S. Chemical regulation and the hormone concept. Biol. Rev. 101:427-41:1935.

18. IMMER, F.R. and HAYES, H.K. Statistical determination of barley varietal adaptation. Jour. Am. Socy. Agron. 26(5):---: May 5, 1934.

19. KONINGSBERGER, V.J. Private communication.

20. KRAUS, E.J., BROWN, N.A. and HAMNER, K.C. Histological reactions of bean plants to indoleacetic acid. Bot. Gaz. 98(2): 370-420: Dec. 1936.

21. KUPFER, E. Studies in plant regeneration. Memoirs of the Torrey Bot. Club. 12:195-241: June 10, 1907.

22. LA RUE, C.D. Regeneration in monocotyledonous seedlings. Am. Jour. Bot. 22(4) :486-92: 1935.

23. LEONIAN, L.H. and LILLY, V.G. Is heterauxin a growth-promoting substance? Am. Jour. Bot. 24(3) : 135-39: 1937.

24. LILLY, V.G. Growth substances for fungi. Proc. West Virginia Acad. Sci. 10:95-103 : 1936.

25. LOEB, J. Rules and mechanisms of inhibition and correlation in the regeneration of Bryophyllum calycinum. Bot. Gaz. 60:249-77: July-Dec., 1915.

26. ----- Further experiments on correlation of growth in Bryophyllum calycinum. Bot. Gaz. 62:293-302: 1916.

27. ----- Influence of the leaf upon root formation and the possibility of a hormone theory of these processes. Bot. Gaz. 63:25-50: Jan.-June, 1917.

28. MACHT, D.I. and GRUMBEIN, M.L. Influence of indoleacetic, indole-butyric, and naphthalene-acetic acids on roots of Lupinus albus seedlings. Am. Jour. Bot. 24(7): 457-61: 1937.

29. MARMER, D.R. Growth of wheat seedlings in solutions containing chemical growth substances. Am. Jour. Bot. 24(3): 139-45: 1937.

30. McCALLUM, W.B. Regeneration in plants. Bot. Gaz. 40:97-120: July-Dec., 1905.

31. MEESTERS, A. The influence of heteroauxin on the growth of root-hairs and roots of Agrostomma gythago L. Bot. Lab. Amsterdam. Proc. 39(1): 91-97: 1936.

32. MORGAN, T.H. The hypothesis of formative stuffs. Bull. Torrey Bot. Club 30(4): 206-13: 1903.

33. MULLER, A.M. The influence of auxin from Orchid pollinia and from urine upon the growth of axillary buds. Biol. Abstracts 11(7): 15683: pp. 1675: August-October, 1937.

34. PFEIFFER, N.E. Anatomical study of root formation on application of indolebutyric acid to Cissus aerial roots. Contrib. Boyce Thompson Inst. 8(6): 493-506: 1937.

35. REA, H.E. and KARPER, R.E. Propagating sorghums by cuttings. Am. Jour. Bot. 19(6): 467-77: 1932.

36. ROBBINS, W.J. and JACKSON, R. Effect of acetic acid on cell walls of stems and roots.

37. SCHEER, B.A. Straight growth of the avena coleoptile in relation to different concentration of certain organic acids and their potassium salts.

38. SNOW, R. Growth regulators in plants. The New Phytologist, 31(4): 336-54: 1932.

39. THIMANN, K.V. and BONNER, J. The mechanism of the action of the growth substance of plants. Proc. Nat. Acad. Sci. 18:30-46, 692-701: 1932.

40. THIMANN, K.V. Auxin and the growth of roots. Am. Jour. Bot. 23:561-69: 1936.

41. ----- and BONNER, J. The inhibition action of the growth substance on bud development. Proc. Nat. Acad. Sci. 19:714-16: 1933.

42. ----- Growth substances in plants. Ann. Rev. Biochem. 4:545-68: 1935.

43. ----- The physiology and chemistry of the plant hormones. Current Science. 4(10):716-21: 1936.

44. ----- On the physiology of the formation of nodules on legume roots. Proc. Nat. Acad. Sci. 22:511-14:1936.

45. ________ On the nature of inhibitions caused by auxin. Am. Jour. Bot. 24 (7) : 407-12 1937.

46. ________ Private communication. 1937, 1938.

47. TRURECKAJA, R. K. On the influence of heterauxin on the root formation in perennial plants. Comptes Rendus (Doklady) de L'academie des Sciences de L'U.S.S.R. 17 (3) 143-45 : 1937.

48. VAIDYANATHAN, R. B. M. Application of statistics to field technique in agriculture. Current Science 4 (7) : 457-68:1936.

49. VAN DER LEK, H-A. A. Anatomical structure of woody plants in relation to vegetative propagation. 9th International Hort. Cong. London: 66-76 August 7-15 : 1930.

50. WENT, F. A. F. G. The investigation on growth and tropisms carried on in the Bot. Lab. of the University of Utrecht during the last decade. Viol. Rev. 10 (2) : 187-207 : 1935.

51. WENT, F. W. Auxin - the plant growth hormone. Bot. Rev. 1 (4) : 162-82 : 1935.

52. ________ Transplantation experiments with peas. Am. Jour. Bot 25 (1) : 44-56:1938.

53. ________ Specific factors other than auxin affecting growth and root formations. Plant Physiology 3 (1) : 55-81 : 1938.

54. ________ and Thimann, K. V. Phytohormones. The Macmillan Co., New York : pp. 294 : 1937.

55. ________ BONNER, J. and WARNER, G. C. "Aneurin and the rooting of cuttings. Dcience 87 (2251) : 17071 Feb. 18, 1938.

56. WINTER, CLARA WOLFANGER; Vascular system of young plants of Medicago Sativa. Bot. Gaz 94 (1) : 152-67 : 1932.

57. ________ ZIMMERMANN, P. W. Oxygen requirements for root growth of cuttings in water.

58. ________ and HITCHCOCK, A. E. The response of roots to root forming substances, etc. Contrib. Boyce Thompson Inst., Vol. 7 and 8, 1935,

59 ________ Responses of plants to hormone-like substances. Ohio Jour. Sci. 37 (6) : 333-48 : Nov., 1937.

60. ________ Private communication.

APPENDIX I.

The effect of B-indolylbutyric acid (acid 3) on the rooting of alfalfa cuttings made at the early blossom stage when grown in a mixture of sand and peat for 22 day

Treatment (hours)	Concentration 300 p.p.m		200 p.p.m.		150 p.p.m.		100 p. p.m.		50 p.p.m.		Control	
	No. planted	No. rooted	No. planted	No. rooted	No. planted	No. rooted	No. planted	No. rooted	No. planted	No. rooted	No. planted	No. rooted
5	10	0	10	5	10	3	10	0	10	0	10	0
10	10	0	10	3	10	0	10	0	10	0	10	1
15	10	0	10	0	10	3	10	5	10	1	10	0
20	10	0	10	0	10	6	10	5	10	0	10	2
30	10	5	10	5	10	0	10	10	10	2	10	4
Totals	50	5	50	13	50	11	50	20.	50	3	50	7

APPENDIX II.

The effect of B-indolylacetic acid (acid 3) on the rooting of alfalfa cuttings made at the early blossom stage when grown in sand for 22 days.

Treatment (hours)	Concentration										Control	
	300 p.p.m.		200 p.p.m.		150 p.p.m.		1000p.p.m.		50 p.p.m.			
	No. Pltd.	No. rtd.	No. Pltd.	No. rtd.	No. pltd.	No. rtd.	No. Pltd.	No. rtd.	No. Pltd.	No rtd.	No. Pld	No. rtd
5	4	2	4	0	4	3	4	1	4	3	4	0
10	4	0	4	2	4	4	4	1	4	0	4	0
15	4	0	4	2	4	1	4	2	4	0	4	2
20	4	0	4	0	4	3	4	2	4	0	4	0
30	4	0	4	0	4	3	4	1	4	3	4	3
Totals	20	2	20	4	20	14	20	7 Y	20	6	20	5

APPENDIX III

Effect of B-indolylbutyric acid (acid 2) on alfalfa cultings made at the early blossom stage when grown in a mixture of sand and peat, also in sand alone.

Medium	Treatment (hours)	6										Control	
		300 p.p.m.		200p.p.m.		150 p.p.m.		100 p.p.m.		50 p.p.m.		p.p.m.	
Sand and Peat		No. Pltd.	No. rtd.	No. Pld.	No. rtd.	No. Pd.	No. rtd.	No. pltd	no. rtd.	No. Pltd.	No. rtd.	No. Pd.	No. Rtd
	5	2	1	3	1	3	1	2	1	2	2	2	0
	10	2	0	3	1	3	0	2	1	2	1	2	0
	15	2	0	2	1	2	0	2	1	2	1	3	1
	20	2	0	2	1	2	0	2	1	2	1	3	0
	30	2	0	2	0	2	0	2	1	2	1	3	1
	Totals	10	1	12	4	12	1	10	5	10	7	13	2
Sand	5	3	0	2	1	4	3	3	2	2	2	3	3
	10	4	0	2	2	3	3	3	0	2	0	3	2
	15	4	0	2	2	3	2	3	1	3	2	3	1
	20	4	0	3	1	3	1	3	0	3	3	3	1
	30	4	0	2	0	3	0	3	0	3	3	3	0
	Totals	19	0	10	6	16	9	15	3	13	10	15	7

APPENDIX IV.

The effect of lower concentrations of indolylacetic and indolylbutyric acids (3 and 2 respectively) on rooting of alfalfa cuttings grown in a sand-peat mixture and in sand alone.

Media	Stage of the parent plant	Acid	Concentration	Treatment (hours)	No. of cuttings planted	No. rooted
Sand and Peat	Early flowering	Indolylacetic	20 p.p.m.	16	2	2
		"	20 p.p.m.	24	2	2
		"	10 p.p.m.	16	2	1
		"	10 p.p.m.	24	2	0
"	"	"	5 p.p.m.	16	2	0
		"	5 p.p.m.	24	2	0
		Control	______	24	2	1
	"	Indolylbutyric	20 p.p.m.	16	2	2
		"	20 p.p.m.	24	2	1
		"	10 p.p.m.	16	2	0
		"	10 p.p.m.	24	2	2
		"	5 p.p.m.	16	2	0
"	"	"	5 p.p.m.	24	2	0
		Control	______	16	2	1
				Totals	28	12
Sand	Before flowering	Indolylacetic	40 p.p.m.	2 days	6	6
	"		40 p.p.m.	3 "	6	2
	"		40 p.p.m.	4 "	6	6
"	Young and tender	Control	______	2	6	3
		Indolylbutyric	40 p.p.m.	2 "	6	6
"	Early flowering	"	40 p.p.m.	3 "		6
			40 p.p.m.	3 "		6
		Control	______			4
				Totals	48	39

APPENDIX V.

Effect of L-naphthaleneacetic acid (acid 1) on the rooting of alfalfa cuttings grown in sand.

Stage of the parent plant	Concentration	Treatment	No. of cuttings planted	No. rooted
Fairly mature	30 p.p.m.	20 hours	5	5
	0 p.p.m.	3 days	5	1
	15 p.p.m.	20 days	3	2
	15 p.p.m.	3 days	4	4
	Control.	20 hours	5	3
		Totals	22	15

Effect of B-indolylbutyric acid (acid 2) and L-naphthaleneacetic acid (acid 1) on the rooting of one-node cuttings.

Stage of the parent plant	Acid	Concentration	Treatment (hrs)	No. of cuttings planted	No. rooted
Heading	B-indolylbutyric	50 p.p.m.	24	10	0
"	L-naphthalene-acetic acid	30 p.p.m.	24	10	0
"	Contro.	______	24	10	0

APPENDIX VI.

A The effect of indolylacetic and indolylbutyric acid on the rooting of cuttings* made a few days after heading and grown in sand.

Media	Acid	Concentration	Treatment (hours)	No. of cuttings planted	No. rooted
Sand	Indolylacetic	20 p.p.m.	16	2	0
		20 p.p.m.	24	2	0
		Control	16	2	0
		10 p.p.m.	16	2	1
		10 p.p.m.	24	2	1
		5 p.p;m.	24	2	0
	Indolylbutyric	20 p.p.m.	16	2	0
		20 p.p.m.	24	2	0
		10 p.p.m.	16	2	1
		10 p.p.m.	24	2	1
	Control	———	16	2	0
A MIXture of sand and peat	Indolylacetic	40 p.p.m.	2 days	4	0
		40 p.p.m.	4 "	4	0
		40 p.p.m.	2 "	4	1
		40 p.p.m.	4 "	4	0
	Control	———	2 "	4	0

B Effect of L-naphthaleneacetic acid on the rooting of one-node cuttings made a few days after heading, grown in the sand and peat mixture.

Treatment	50 p.p.m.		25 p.p.m.		15 p.p.m.		Control	
	No. planted	No. rooted	No. planted	NO. rooted	No. planted	No. rooted	No planted	NO root
20 hours	4	0	4	1	4	0	4	0
2 days	4	0	4	0	4	0	4	0
3 days	4	0	4	0	4	1	4	0
4 days	4	0	4	0	4	0	4	0

* **Wheat**

APPENDIX VII

Preparation of Nutrient Solution

The solution was made up from the stock solutions as follows:----

Ca $(No_3)_2$	M/1	5c.c.
KNO_3	M/1	5 c.c.
$MgSO_4$	M/1	2 c.c.
KH_2PO_4	M/1	1 c.c.
Ferric tartrate	.5%	1 c.c.
		14 c.c.

The volume was brought up to one litre by the addition of distilled water.

APPENDIX VIII

The actual number of roots visible, after 22 days treatment has been applied as a measure of the effectiveness of the auxin treatment.

Acid		Plant 1: 1	2	3	4	Total	Plant 2: 1	2	3	4	Total	Plant 3: 1	2	3	4	Total	Plant 4: 1	2	3	4	Total	Total	Mean
-----	Check Bottom	9	0	12	9	30	0	0	6	7	13	12	0	3	12	27	6	12	9	5	32	102	6.38
L -Naphthaleneacetic	30 p.p.m. "	13	0	16	0	29	6	14	11	0	31	16	1	16	5	38	9	8	13	7	37	135	8.40
"	50 p.p.m. "	0	0	0	0	0	0	0	15	0	15	0	1	0	1	2	15	12	17	15	59	76	4.80
B - Indolylbutyric	30 p.p.m. "	22	15	10	14	61	0	17	13	7	37	0	10	13	0	23	14	8	13	11	46	167	10.40
"	50 p.p.m. "	0	17	17	12	46	0	1	14	15	30	0	20	14	2	36	11	8	10	11	40	152	9.50
-----	Check Top	18	14	21	13	66	12	15	15	17	59	19	30	20	15	67	13	12	14	9	48	240	15.00
L -Naphthaleneacetic	30 p.p.m. "	0	18	18	0	36	26	29	24	26	105	16	23	29	2	70	26	29	14	14	83	294	18.40
"	50 p.p.m. "	19	0	17	14	50	9	23	12	14	58	1	1	9	0	11	23	20	17	14	74	193	12.10
B - Indolylbutyric	30 p.p.m. "	22	19	29	20	90	25	28	25	29	107	17	12	19	16	64	14	20	17	14	65	326	20.40
"	50 p.p.m. "	27	28	26	17	98	29	28	22	23	102	13	9	11	14	47	16	13	16	17	62	309	19.30
Totals		130	111	166	99	506	107	155	157	138	557	94	80	134	67	383	147	142	140	117	546	1994	

$N = 160$

$E(X) = T = 1994$

$T^2 = 3976036$

$E(X^2) = 35996$